LODISH et al. (1995) MOL CELL BIOL. 3rd ED., CHAPTER 15, p. 389-396.

Exhibit A

TABLE 10-4 Human Hereditary Diseases Associated with DNA-repair Defects

Disease*	Sensitivity	Cancer Susceptibility	Symptoms
Ataxia telangiectasia	γ irradiation	Lymphomas	Ataxia, dilation of blood vessels in skin and eyes, chromosome aberrations, immune dysfunction
Bloom's syndrome	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Cockayne's syndrome	UV irradiation		Dwarfism, retinal atrophy, photosensitivity, progeria, deafness, trisomy 10
Fanconi's anemia	Cross-linking agents	Leukemias	Hypoplastic pancytopenia, congenital anomalies
Xeroderma pigmentosum	UV irradition, chemical mutagens	Skin carcinomas and melanomas	Skin and eye photosensitivity, keratoses

*Other human hereditary disorders that may be related to DNA-repair defects include dyskeratosis congenita (Zinsser-Cole-Engman syndrome), progeria (Hutchinson-Gilford syndrome), and trichothiodystrophy.
SOURCE: From A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 788.

product encoded by the yeast *RAD14* gene has considerable homology with the protein encoded by one of the genes mutated in xeroderma pigmentosa patients. In addition, DNA transfection experiments have revealed that certain human genes can rescue some UV-sensitive CHO cell mutants. Two of the human genes identified in this way have been shown to be related to the yeast *RAD3* and *RAD10* genes. The human protein with partial homology to the RAD10 protein also contains a region that is similar to part of *E. coli* UvrC.

Recombination between Homologous DNA Sites

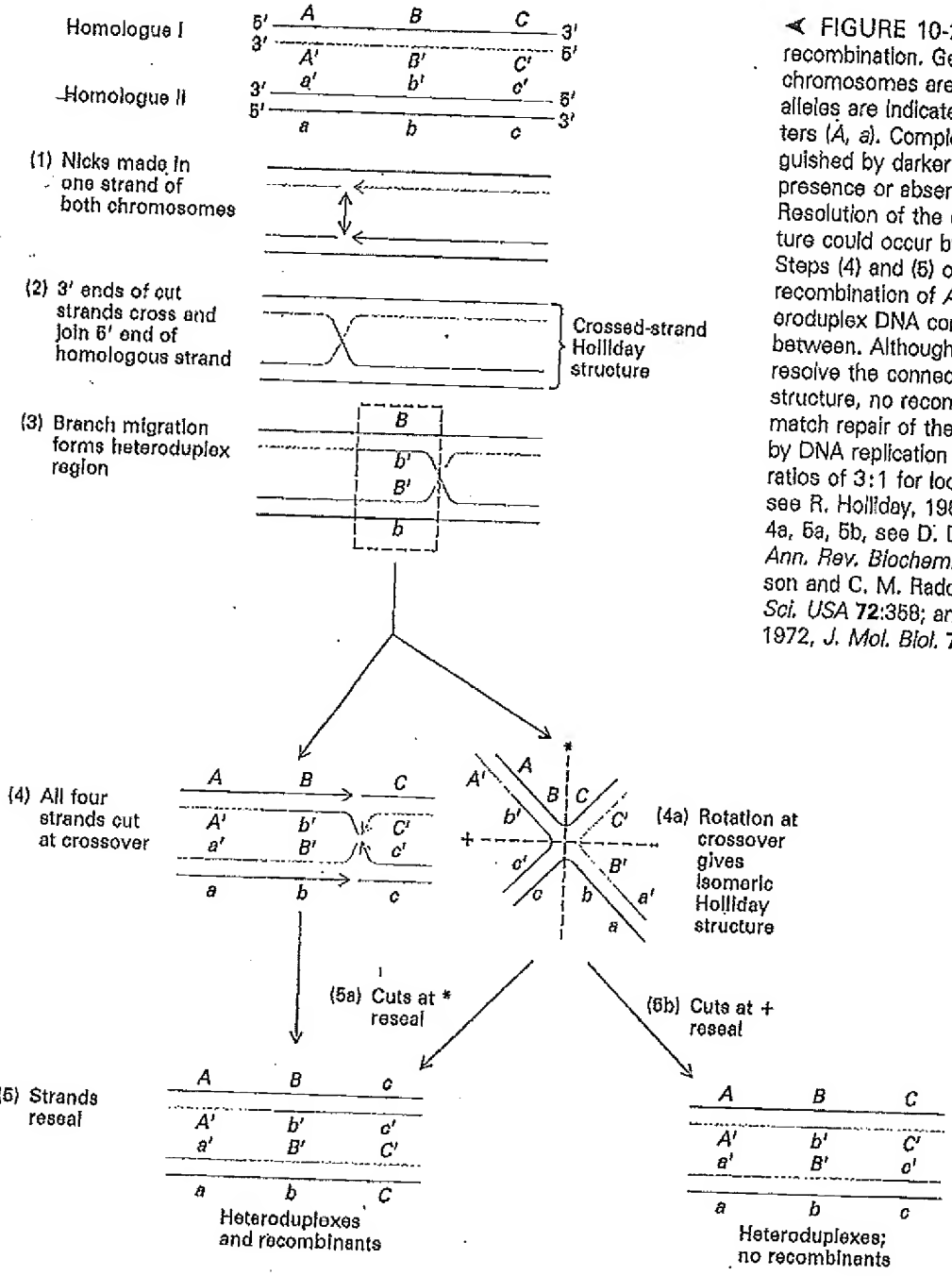
In the previous sections of this chapter, we discussed the enzymatic mechanisms by which the genome is faithfully reproduced from one generation to another through the process of DNA replication, and the phenomenon of DNA repair necessary to maintain the correct DNA sequence. In this section, we examine the mechanisms of recombination by which the genome can change from one generation to another.

Soon after Mendel's rules of independent gene segregation were rediscovered and the segregation of linked groups of genes on individual chromosomes was widely recognized, another great genetic discovery was made in *D. melanogaster*: blocks of genes from homologous chromosomes could be exchanged by the process of crossing over, or *recombination*. Recombination, which takes place during meiosis in sexually reproducing organisms, provides a mechanism for generating genetic diversity beyond that achieved by the independent assortment of chromosomes. Genetic exchange by recombination occurs not only in animals and plants but also in prokaryotes, viruses, plasmids, and even in the DNA of cell organelles such as mitochondria.

The events in a *reciprocal recombination* are equivalent to the breakage of two duplex DNA molecules representing homologous but genetically distinguishable chromosomes, an exchange of *both* strands at the break, and a resolution of the two duplexes so that no tangles remain. The frequency of recombination between two sites is proportional to the distance between the sites. (As discussed in Chapter 8, this phenomenon is the basis of genetic mapping of genes defined by mutations.) In the remainder of this chapter, we describe various proteins involved in recombination and models of how they carry out this process.

Holliday Recombination Model Is Supported by Observation of Predicted Intermediate Structures

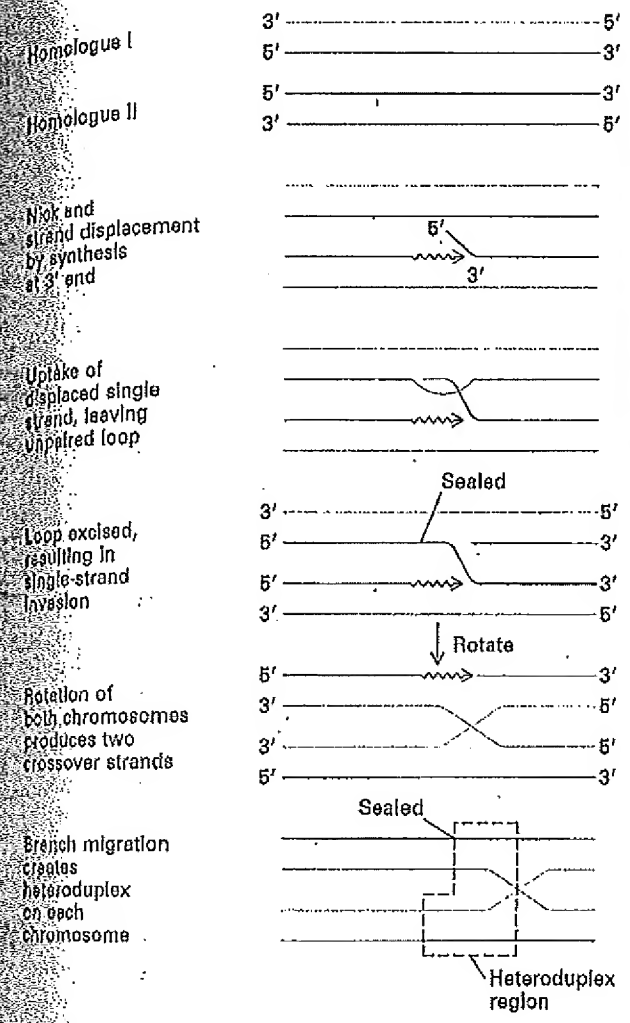
A proposal by Robin Holliday in 1964 is the basis of the most popular current models for the molecular events of recombination, which are illustrated in Figure 10-28. In step 1, a nick is made in one strand of each of the two homologous chromosomes that are going to recombine. Strand exchange then occurs at the site of the nicks, and



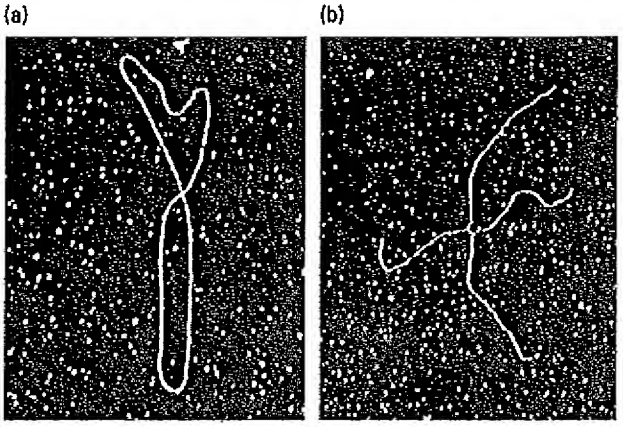
◀ FIGURE 10-28 Holliday model of genetic recombination. Genetically distinct homologous chromosomes are indicated by red and blue; alleles are indicated by capital and lowercase letters (*A*, *a*). Complementary strands are distinguished by darker and lighter shades and by the presence or absence of prime signs (*A*, *A*′; *a*, *a*′). Resolution of the crossed-strand Holliday structure could occur by two different pathways. Steps (4) and (5) or (4a) and (5a) would result in recombination of *AC*/*ac* to give *Ac*/*aC* with heteroduplex DNA containing the *B* locus in between. Although steps (4a) and (5b) also resolve the connected strands of the Holliday structure, no recombinants are produced. Mismatch repair of the heteroduplex region followed by DNA replication would lead to segregation ratios of 3:1 for loci in this region. [Steps 1–5, see R. Holliday, 1964, *Genet. Res.* **5**:282; steps 4a, 5a, 5b, see D. Dressler and H. Potter, 1982, *Ann. Rev. Biochem.* **51**:727; also see M. Meselson and C. M. Radding, 1975, *Proc. Nat'l Acad. Sci. USA* **72**:358; and N. Sigal and B. Alberts, 1972, *J. Mol. Biol.* **71**:769.]

the cut 3′ ends are joined to the 5′ ends of the homologous strand, producing a *crossed-strand Holliday structure* (step 2). The branch point then migrates, creating a *heteroduplex* region containing one strand from each parental chromosome (step 3).

Two mechanisms have been proposed for separation, or *resolution*, of the connected duplexes. According to the original proposal, all four strands are cut at the crossover site and the left side of chromosome I joins the right side of chromosome II, and vice versa (steps 4 and 5 in Figure 10-28). Both strands in each of the resulting duplexes are recombinant; that is, all markers to the left and right of the crossover site have undergone reciprocal recombination. A later proposal simplifies the enzymatic cutting that is necessary to resolve the crossed-strand intermediate. Rotation of the Holliday structure at the crossover site forms a rotational isomer, or isomeric *Holliday structure* (step 4a). The two connected duplexes of this structure could be resolved



▲ FIGURE 10-29 Meselson-Radding model of genetic recombination. Genetically distinct homologous chromosomes are indicated by red and blue; complementary strands are distinguished by darker and lighter shades. In this modification of the Holliday model, formation of the crossed-strand Holliday structure begins with a nick in one of the chromosomes. After rotation at the crossed-strand site and branch migration of the crossed-strand intermediate, resolution would occur as shown in Figure 10-28. [See M. Meselson and C. Radding, 1975, *Proc. Nat'l. Acad. Sci. USA* 72:358.]



▲ FIGURE 10-30 Electron micrographs of plasmid DNA in the process of recombination. (a) Circular plasmid DNA in crossed-strand Holliday structure. (b) More highly magnified view reveals single-stranded ring in center of isomeric Holliday structure that results from rotation about the crossover point. [See H. Potter and D. Dressler, 1978, *Cold Spring Harbor Symp. Quant. Biol.* 43:969; courtesy of D. Dressler.]

by cutting and rejoining of only two strands. If this involves the two strands that were not cut to generate the original Holliday intermediate, then recombinant duplex chromosomes containing a heteroduplex region are produced (step 5a). However, if resolution involves cutting of the two strands that were originally cut, the resulting duplex chromosomes contain a heteroduplex region but are not recombinants (step 5b).

In a further modification of the Holliday recombination model, Matthew Meselson and Charles Radding suggested another mechanism for creating a crossed-strand Holliday structure; this mechanism requires a single-strand cut in only one chromosome (Figure 10-29). DNA synthesis from the nick leads to the formation of a 5′-phosphate end, which then invades the homologous duplex DNA. After branch migration of the crossed-strand region, rotation and resolution produce results similar to those described in Figure 10-28. As we will discuss in the next section, this model is supported by the finding that a single strand can be used to initiate a recombination event using a single *E. coli* protein in vitro.

Do Holliday intermediates actually exist? Viral and plasmid DNA molecules in the act of recombining can be extracted from both bacterial and animal cells. Electron micrographs of such molecules have revealed structures similar to the crossed-strand and isomeric Holliday structures (Figure 10-30). Thus, regardless of the mechanism initiating recombination, the final connection between the unresolved chromosomes seems to involve branch migration and chromosomal rotation.

Recombination in *E. coli* Occurs by Three Similar Pathways That All Require RecA Protein

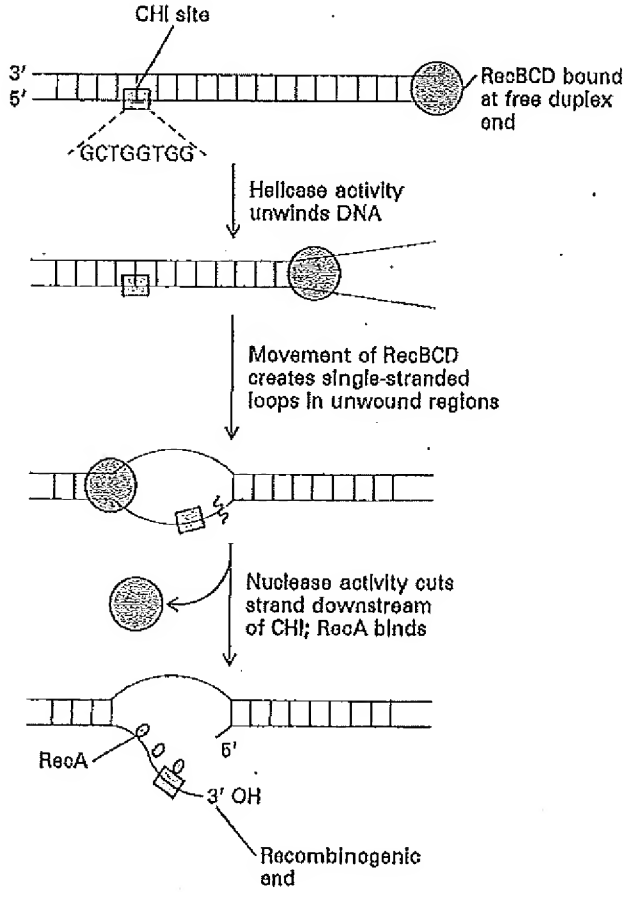
Genetic analysis has revealed three different pathways of recombination in *E. coli*. Not only is the RecA protein required in all three pathways, but they also share the following steps: (a) generation of a single-stranded DNA segment

with a 3′-hydroxyl end; (b) invasion of the duplex by the 3′ *recombinogenic* end of the single-stranded DNA and complexing with regions of homology; (c) formation of a Holliday structure, which can undergo branch migration; and (d) endonuclease cleavage followed by ligation to yield recombinants. Recombination in *E. coli*, as in the Meselson-Radding model, is initiated by a single-strand cut; however, in *E. coli* the recombinogenic end is a 3′-hydroxyl end, whereas in the Meselson-Radding model it is a 5′-phosphate end (see Figure 10-29). In this section, we describe the primary *E. coli* recombination pathway, which is initiated by an enzyme complex called RecBCD.

Initiation of Recombination (RecBCD Enzyme)

The most common way that *E. coli* cells generate a recombinogenic single-stranded region of DNA probably is by the action of the RecBCD enzyme. This enzyme complex, which is composed of proteins encoded by the *recB*, *C*, and *D* genes, specifically recognizes double-strand breaks. Such breaks occur naturally during bacterial conjugation, a process in which chromosomal DNA is transferred from one bacterium to another through direct cell contact. Double-stranded breaks also can be generated by exposure to x-rays and certain chemicals.

The mechanism of action of RecBCD was worked out in studies with bacteriophage λ, which has a linear DNA genome with free blunt ends equivalent to double-strand breaks. Certain regions of λ-phage DNA undergo recombination at higher frequencies than other regions in normal *E. coli* host cells but not in *recBCD* mutant host cells. The sites of increased recombination were named *CHI sites* because the Greek letter chi looks like a crossover point. Experiments with purified RecBCD enzyme and λ DNA indicate that the protein recognizes and binds to a free end of the λ-phage chromosome. The enzyme then functions as a helicase, moving along and remaining attached to the duplex DNA as it unwinds it (Figure 10-31). Because RecBCD enzyme unwinds the DNA faster than it is rewound, single-stranded loops are created as the protein progresses. After a CHI site has been passed, a specific nuclease activity of RecBCD cuts one of the exposed strands, leaving a free 3′-hydroxyl end just downstream from the CHI site. This end can now participate in the process of strand invasion, which is catalyzed by RecA protein.

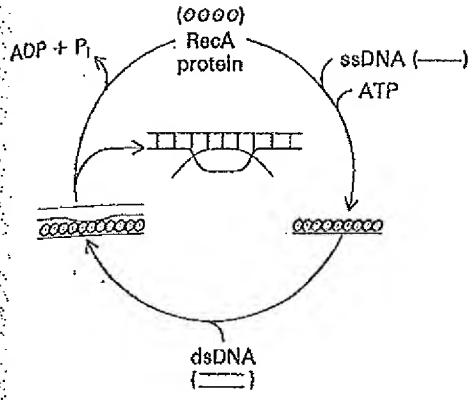


▲ FIGURE 10-31 Initiation of recombination by *E. coli* RecBCD enzyme. This enzyme, which has both helicase and nuclease activities, produces a single-stranded DNA region with a free 3′-hydroxyl end. This recombinogenic end binds RecA, which catalyzes strand invasion and formation of a Holliday structure. [See A. F. Taylor et al., 1985, *Cell* 41:153.]

Strand Invasion, Homologous Pairing, and Formation of Holliday-Type Structure (RecA Protein)

Studies with single-stranded DNA (ssDNA) showed that the protein encoded by the *recA* gene can bind to ssDNA. In the presence of target duplex DNA, the RecA-ssDNA complex can find and bind to a target-DNA region homologous to the ssDNA. RecA then can insert the ssDNA into the target DNA, displacing one of the preexisting strands and forming a heteroduplex Holliday-type structure (Figure 10-32).

In subsequent studies, RecA was shown to bind to ssDNA regions generated by action of the RecBCD enzyme. *E. coli* Ssb protein stimulates this reaction by binding to the single-stranded region and preventing intrastrand base pairing, which would inhibit binding of RecA. In the presence of ATP, RecA coats the single-stranded region and polymerizes, forming a filament that wraps around the entire length of the ssDNA. Because the polymerization of RecA occurs in the 5′ → 3′ direction along the DNA, coating takes place in a discontinuous fashion as a region of the duplex is unwound. X-ray crystallographic analysis of RecA suggests that each molecule has two DNA-binding sites, both of which may lie within the core of the filament.

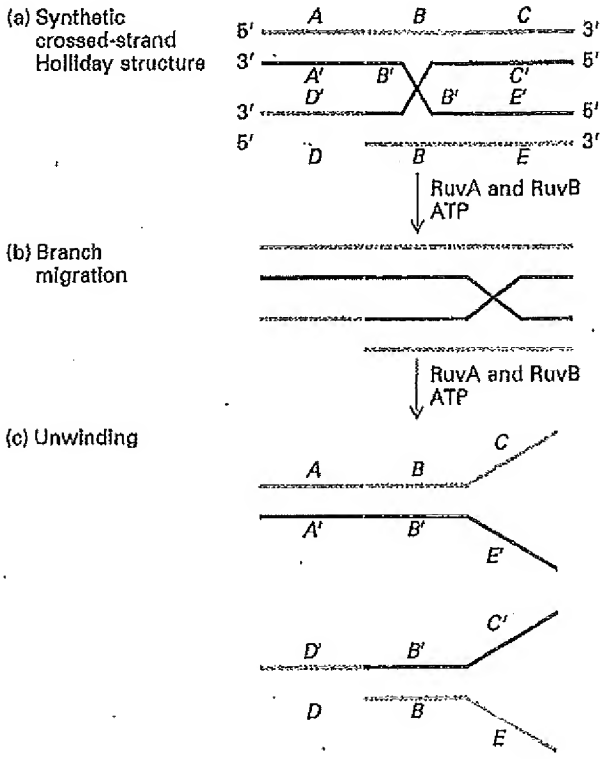


A FIGURE 10-32 Formation of Holliday-type structure by *E. coli* RecA. In the presence of ATP, RecA binds single-stranded DNA (ssDNA) and promotes insertion of the bound strand at a homologous region of double-stranded DNA (dsDNA), yielding a crossed-strand Holliday-type structure. The insertion reaction requires the ATPase activity of RecA. [See S. S. Flory et al., 1984, *Cold Spring Harbor Symp. Quant. Biol.* 49:513.]

RecA effectively catalyzes the in vitro formation of Holliday structures between a double-stranded circular DNA containing a short single-stranded gap and a fully double-stranded linear DNA. Although formation of Holliday structures depends on RecA, their maintenance does not. Removal of RecA leaves stable Holliday structures, which have been used as substrates in studies on branch migration and resolution.

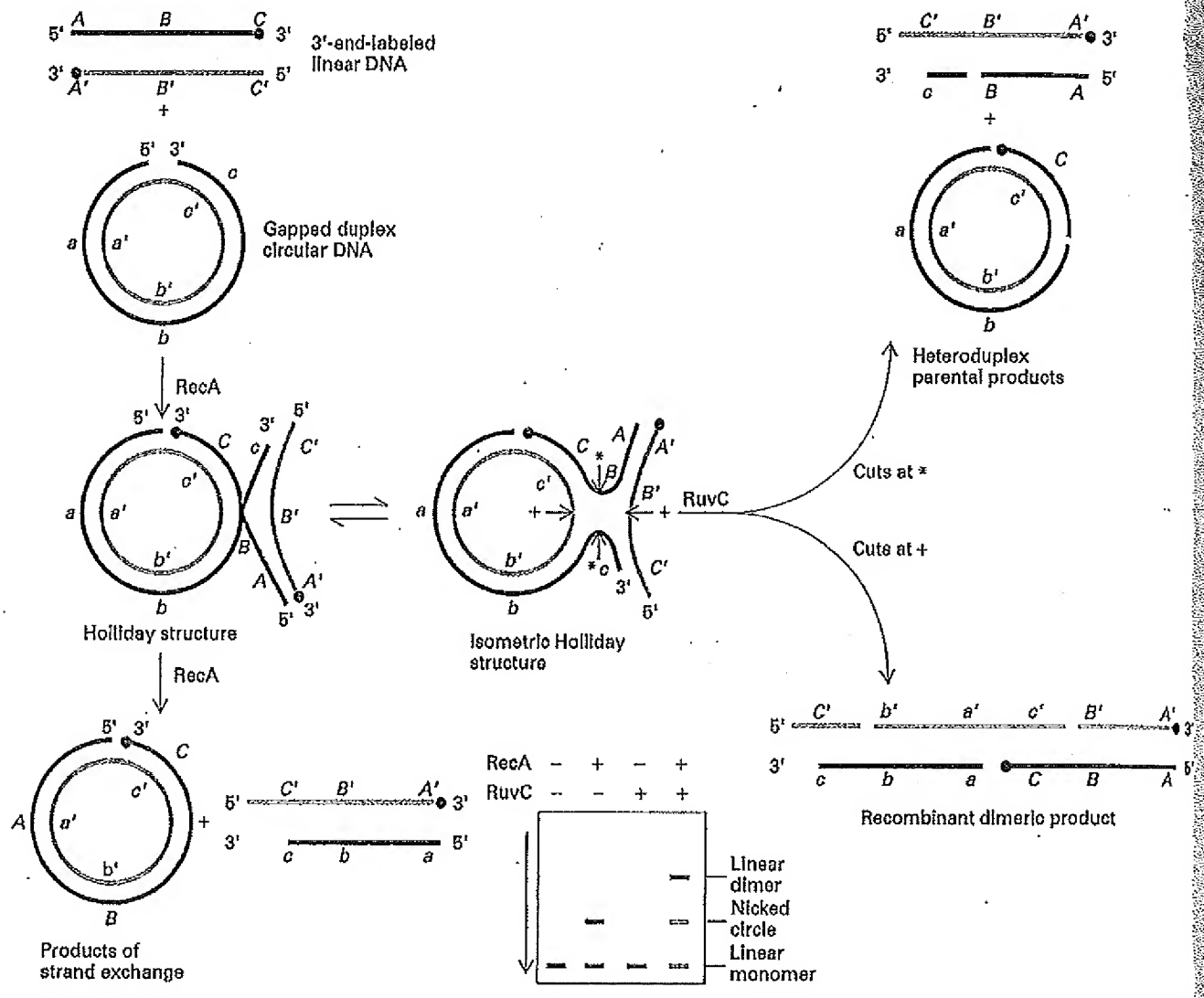
RecA protein also is important in DNA repair, in part, through its central role in regulating the *SOS response* of *E. coli* to UV irradiation. When *E. coli* cells are irradiated with UV light, a whole series of *SOS* genes that enable the bacterium to survive is activated, including the *recA* gene itself. In irradiated cells, the RecA protein promotes the proteolytic breakdown of various DNA-binding proteins. For instance, breakdown of the lambda cI repressor is dependent upon *recA* function; this inactivation of the repressor releases the dormant phage in irradiated cells. RecA protein does not appear to be a protease itself, but rather promotes the DNA-binding proteins to degrade themselves in a reaction requiring ATP and single-stranded DNA (in vivo the single-stranded DNA is probably formed by DNA breakdown induced by irradiation).

RecA protein also promotes the cleavage of LexA protein. This protein, the product of the regulatory gene *lexA*, is a repressor of a series of other genes, many of which are involved in DNA repair. For instance, both the *recA* gene itself and the genes for the UvrABC nuclease are expressed when the LexA protein is digested. The elaborate SOS response sheds light on why UV irradiation not only induces repair synthesis but also, because of the increased RecA production, increases recombination.



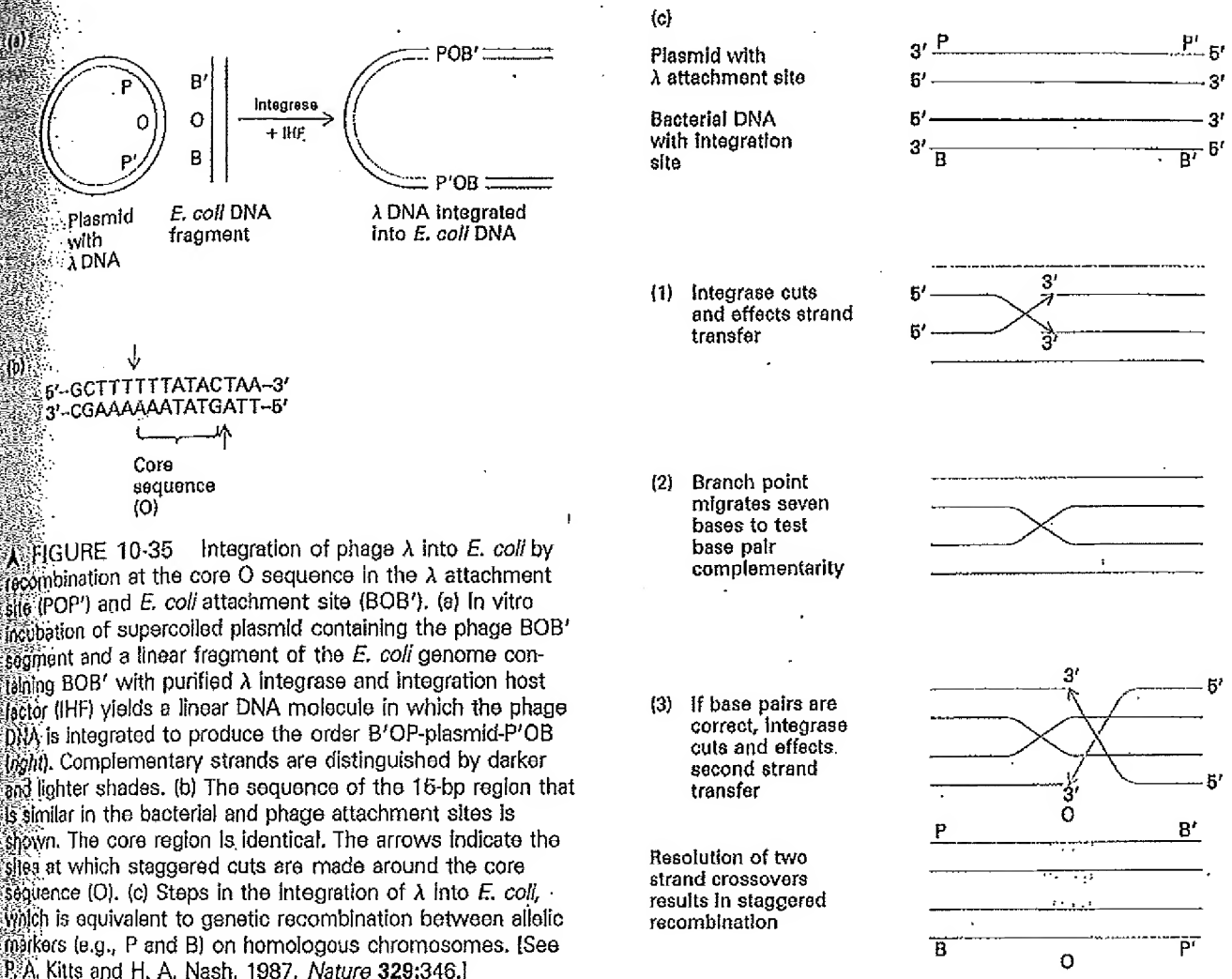
A FIGURE 10-33 Experimental demonstration of branch migration catalyzed by *E. coli* RuvA and RuvB proteins. A synthetic Holliday structure was produced by annealing synthetic single-stranded oligonucleotides in which only the center *BB′* region was homologous. Complementary strands are indicated by darker and lighter shades and the presence or absence of prime signs (*A,A′;B,B′*); segments with different sequences are indicated by color. (a) In the synthetic Holliday structure, the crossover involves only the homologous region (green). (b,c) Treatment of the Holliday structure with RuvA and RuvB in the presence of ATP leads to branch migration followed by unwinding to yield cruciform structures with nonhomologous single-stranded ends. Branch migration in the other direction (towards *A* and *D*) yields similar cruciform structures. [Adapted from H. Iwasaki et al., 1992, *Genes & Dev.* 6:2214.]

Branch Migration and Resolution of Holliday Structures (Ruv Proteins) Strains of *E. coli* with mutations in the *ruvA*, *ruvB*, or *ruvC* gene exhibit defective recombination, indicating that the proteins encoded by these genes play a role in recombination. Migration of the crossover point in Holliday structures is efficiently catalyzed by RuvA and RuvB. RuvA protein specifically recognizes the Holliday structure, whereas RuvB protein has the helicase activity necessary for promoting branch migration. Studies with synthetic oligonucleotide substrates have clarified the role of these two proteins (Figure 10-33).



A FIGURE 10-34 Demonstration that *E. coli* RuvC protein can resolve Holliday structures. Genetically distinct DNA duplexes are indicated by red and blue; alleles are indicated by capital and lowercase letters (*A,a*). Complementary strands are distinguished by darker and lighter shades and the presence or absence of primes (*A, A'*). A linear DNA molecule end-labeled with ^{32}P (red dot) and a homologous circular DNA molecule containing a short region of single-stranded DNA, called gapped DNA, were incubated with RecA and ATP to produce Holliday structures. In the absence of RuvC, strand exchange catalyzed by RecA yielded two heteroduplex labeled products: a linear monomeric DNA and a nicked circular DNA, corresponding to the parental configuration (i.e., a nicked circle and linear monomer). When RuvC was also present in the reaction mixture, it catalyzed specific cleavage of the Holliday intermediate. Cleavage at + sites would yield a recombinant linear dimer, whereas cleavage at * sites would yield heteroduplex DNAs in the parental configuration. (*Inset*) Diagram of an autoradiograph obtained following gel electrophoresis of the reaction products with a fixed amount of RecA in the presence and absence of RuvC. Note that in the presence of RecA and absence of RuvC, the radiolabel appeared in a linear monomeric DNA and nicked circular DNA. In the presence of RuvC, the primary product detected was a linear dimeric DNA, indicating that RuvC cleaves Holliday structures preferentially at the + sites. [Adapted from H. J. Dunderdale et al., 1991, *Nature* 354:506.]

Holliday structures generated by RecA are efficiently resolved by the nuclease action of the RuvC protein, which binds specifically to these recombination intermediates (Figure 10-34). RuvC only binds to crossed-strand regions that exhibit homology, suggesting that specific base pairing occurs within the crossover region. RuvC is not inhibited



λ FIGURE 10-35 Integration of phage λ into *E. coli* by recombination at the core O sequence in the λ attachment site (POP') and *E. coli* attachment site (BOB'). (a) In vitro incubation of supercoiled plasmid containing the phage BOB' segment and a linear fragment of the *E. coli* genome containing BOB' with purified λ integrase and integration host factor (IHF) yields a linear DNA molecule in which the phage DNA is integrated to produce the order B'OP-plasmid-P'OB (*right*). Complementary strands are distinguished by darker and lighter shades. (b) The sequence of the 15-bp region that is similar in the bacterial and phage attachment sites is shown. The core region is identical. The arrows indicate the sites at which staggered cuts are made around the core sequence (O). (c) Steps in the integration of λ into *E. coli*, which is equivalent to genetic recombination between allelic markers (e.g., P and B) on homologous chromosomes. [See P. A. Kitts and H. A. Nash, 1987, *Nature* **329**:346.]

by the presence of RecA, nor does it require interaction with RecA, as demonstrated by its ability to resolve Holliday structures lacking RecA.

Site-Specific Integration of λ Phages Mimics a Homologous Recombination Event

Several examples of *site-specific* recombination have been discovered in both prokaryotic and eukaryotic cells. Site-specific recombination requires the recognition of unique nucleotide sequences in both DNA molecules by enzymes called *recombinases*, which then catalyze the joining of the two molecules. One well-studied example is the integration of bacteriophage λ into a particular site in the *E. coli* chromosome. Integration can be carried out in vitro with the viral enzyme *integrase*, which in a stepwise fashion makes and then resolves a Holliday junction. Many, but not all, site-specific recombination systems proceed through a Holliday intermediate.

The site-specific integration of λ-phage DNA into the *E. coli* chromosome is thought to be quite similar to generalized homologous recombination. The genome of λ phage contains a 15-bp region, the attachment site, which contains an 7-bp core sequence identical to the integration (or attachment) site in the host-cell DNA. The in vitro integration system consists of a plasmid DNA that contains the phage attachment site (POP'), a linear fragment of the bacterial DNA containing its integration site (BOB'), purified integrase, and a bacterial host protein termed integration host factor (Figure 10-35 a,b). By using DNA molecules with mutations or single-strand breaks in the 15-bp homol-

ogous region, researchers have been able to stop the reaction at several stages and to collect intermediates in the reaction leading to integration.

The interpretation of all the experiments with various mutants is that phage integrase (as a dimer) makes staggered cuts in and catalyzes strand transfer between bacterial and phage DNA at the core sequence of the attachment sites (Figure 10-35c, step 1). As in the case of topoisomerases, the cut ends of the DNA are covalently bound to integrase during this reaction. The resulting strand-transfer structure is identical to the crossed-strand Holliday structure. If the neighboring sequences in the POP′ and BOB′ sites are correct, then a branch migration of seven bases occurs and the duplex remains perfectly paired (step 2). A second strand exchange (step 3) catalyzed by integrase then resolves the Holliday structure, resulting in the integration of the phage DNA into the bacterial chromosome.

Studies in Yeast Are Providing Insights into Meiotic Recombination

A central interest in exploring the mechanisms of homologous recombination in eukaryotes is its role in meiosis. Studies in yeast reveal that defects in meiotic recombination lead to defects in meiosis itself. It is unclear whether these meiotic defects reflect a requirement for meiotic recombination or whether intermediates that accumulate in mutant strains (e.g., double-strand breaks) lead to meiotic arrest. Nonetheless, it seems likely that an understanding of meiotic recombination will be an essential part of our understanding of the complex process of correctly pairing homologous chromosomes during meiosis.

Four different approaches have been taken to studying meiotic recombination in yeast. First, genetic screens have identified one panel of genes required for meiotic recombination and another for both meiotic and mitotic recombination. Second, four proteins have been purified that exhibit a strand-exchange activity similar to that of *E. coli* RecA. The roles of these yeast proteins in recombination remain unclear but can be addressed by gene knockout experiments. Third, analysis of DNA regions undergoing recombination have revealed recombination intermediates containing double-strand breaks with 5′ recessed ends (i.e., 3′ single-stranded tails). Based on this and other observations, a model for yeast recombination has been proposed. And fourth, using a differential hybridization scheme, investigators have identified genes that are selectively expressed during meiosis.

Genetic analysis reveals that *DMC1* (disrupted meiosis, cDNA 1), one of the genes identified by differential hybridization, is required for meiotic recombination in yeast. Yeast cells with mutations in *DMC1* accumulate intermediates that have double-strand breaks and 3′ single-stranded tails. In vitro, *E. coli* RecA protein will bind to this type of intermediate; moreover, RecA and the yeast DMC1 protein exhibit sequence homology. Biochemical studies will be necessary to directly assess whether DMC1 functions in a manner similar to RecA. Interestingly, the protein encoded by the yeast *RAD51* gene has an amino sequence similar to both RecA and DMC1. Mutations in *RAD51* result in defects in both meiotic and mitotic recombination. Based on these very early observations, it is tempting to propose that both prokaryotic and eukaryotic recombination mechanisms may be quite similar.

Gene Conversion Can Occur near the Crossover Point during Reciprocal Recombination

Although markers at some distance from the crossover point are exchanged in a reciprocal fashion during recombination, an apparent nonreciprocal event sometimes occurs at or near the crossover point. This phenomenon is most easily studied in yeast in which each meiotic product can be scored in the haploid progeny spores. In a cross of multiply marked yeast strains that undergo recombination, most allelic markers segregate 2:2, but a few show 3:1 or 1:3 segregation (Figure 10-36). Such a nonreciprocal event is called *gene conversion* because one allele is apparently "converted" into another. It is now known that in gene conversion, the exact base sequence is represented at the converted site, obviously suggesting that exact copying of a DNA strand is involved. This property is referred to as *fidelity* of gene conversion. The occurrence of 1:3 and 3:1 events at equal frequencies is called *parity* of gene conversion.

The double-strand break model of yeast meiotic recombination shown in Figure 10-37 can account for the observed phenomenon of gene conversion. In this model, an intermediate with two crossovers and two heteroduplex regions is generated. The distortion in the heteroduplex regions caused by mispairing of the different alleles (e.g., *D* and *d*) can be recognized by a mismatch repair system, which removes a single-stranded region containing the mismatch and then fills in the gap substituting correctly paired base(s) for the mispaired one(s).

There are three possible results of mismatch repair at the heteroduplex region indicated as *d/D* in Figure 10-37. Conversion of the *d* allele to *D* results in a 3:1 ratio of *D:d* in the spores; conversion of *D* to *d* results in a 2:2 ratio; and no repair results in a 5:3 segregation ratio of *D:d* (Figure 10-38). In the latter case, the heteroduplex remains in the spore; after the first cell division, two daughter cells of different genotypes (reflecting the alleles in the heteroduplex) are generated, giving rise eventually to a sectored colony in which one-half of the colony is phenotypically *D*